

Supplementary Material

Cavitation modulated inflammatory response following focused ultrasound blood-brain barrier opening

Robin Ji^{1*}, Maria E. Karakatsani^{1*}, Mark Burgess¹, Morgan Smith¹, Maria F. Murillo¹,
and Elisa E. Konofagou^{1,2#}

Department of ¹Biomedical Engineering and ²Radiology
Columbia University, New York, New York, USA

*These authors contributed equally.

#Corresponding author

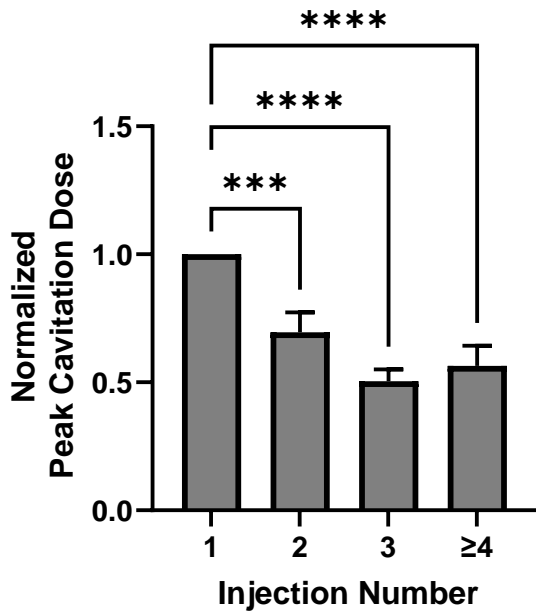


Figure S1. The peak cavitation dose following microbubble injection/reinjections normalized to the peak cavitation dose following the initial injection for each mouse (2 injections: 0.70 ± 0.08 , 3 injections: 0.50 ± 0.05 , 4 or more injections: 0.56 ± 0.08). $N = 7$ mice received at least two injections, $N = 3$ mice received at least three injections, and $N = 8$ mice received four or more injections. Subsequent peak cavitation dose for microbubble bolus injections were significantly lower than the peak cavitation dose from the initial bolus injection (One-way ANOVA with Dunnett's multiple comparisons test). All error bars represent SEM. *** - $p < 0.001$, **** - $p < 0.0001$.

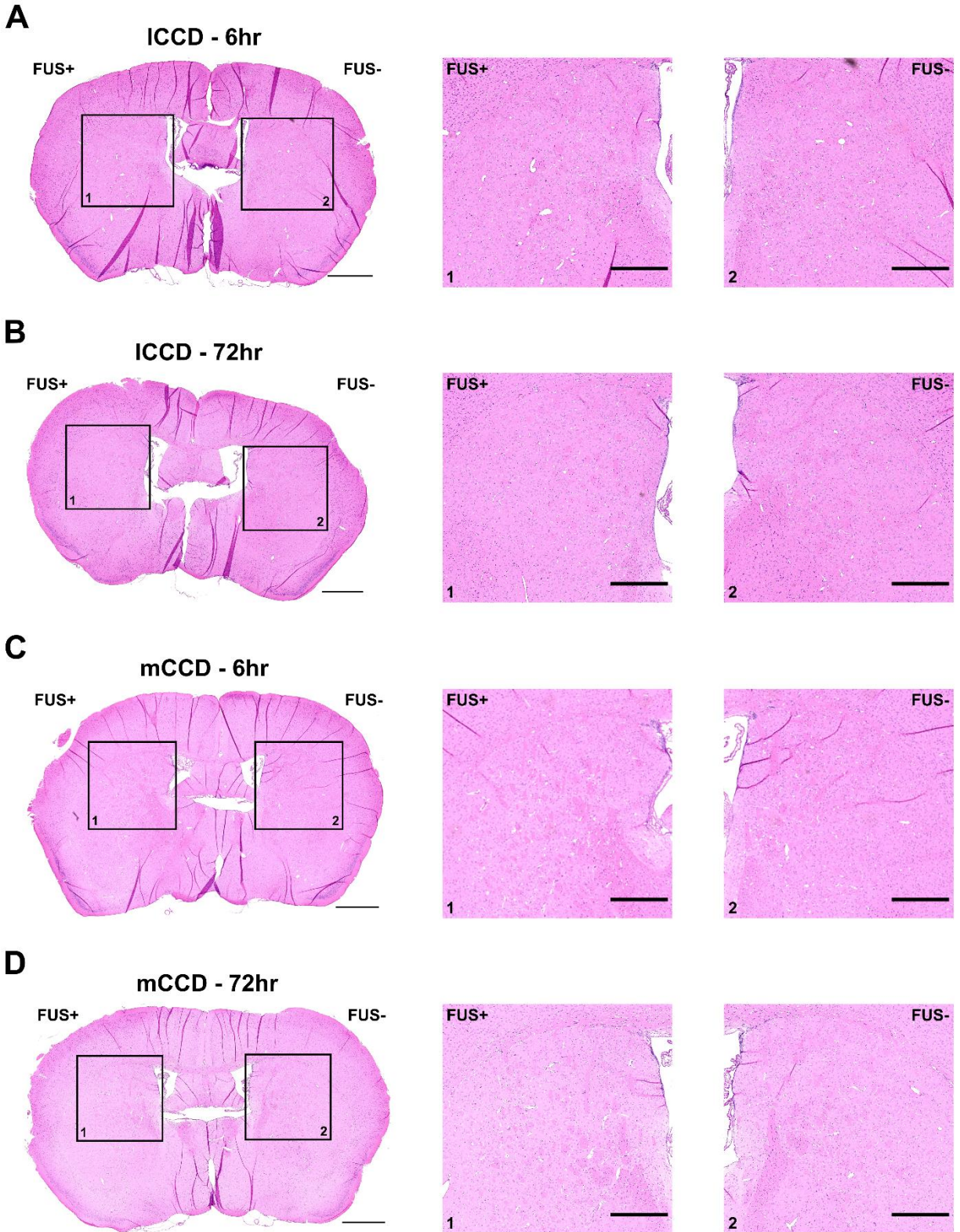


Figure S2. Representative H&E staining for ICCD at (A) 6 hours and (B) 72 hours and mCCD at (C) 6 hours, and (D) 72 hours after BBB opening. H&E staining reveal no significant changes in the treated (FUS+) hemisphere compared to the untreated (FUS-) hemisphere. The scale bar for the full coronal section view is 1 mm and the scale bar for the zoomed in region is 500 μ m

Table S1: Average Log2 fold change for all 84 genes 6 hours after BBB opening. Gene expression is shown for the low, medium, and high CCD groups and are relative to the non-sonicated contralateral region. There are 4 mice per CCD group.

6 Hours After BBB Opening			
Gene Names	ICCD	mCCD	hCCD
Aimp1	0.03	0.00	0.09
Bmp2	0.14	-0.04	-0.10
Ccl1	-0.32	0.32	0.84
Ccl11	-0.23	0.19	1.18
Ccl12	0.78	2.83	3.62
Ccl17	0.06	0.10	0.13
Ccl19	0.21	0.22	0.23
Ccl2	1.27	4.46	5.17
Ccl20	-0.42	0.51	0.52
Ccl22	0.00	-0.03	0.37
Ccl24	-0.32	0.32	0.84
Ccl3	-0.01	1.83	2.12
Ccl4	0.66	2.77	2.84
Ccl5	-0.20	-0.23	0.04
Ccl6	-0.03	0.33	0.21
Ccl7	0.82	5.07	5.00
Ccl8	0.21	0.37	0.89
Ccl9	0.16	0.65	0.62
Ccr1	0.47	0.30	1.18
Ccr10	-0.65	-0.09	0.31
Ccr2	0.72	-0.15	0.75
Ccr3	-0.08	0.69	0.42
Ccr4	-0.32	0.32	0.84
Ccr5	0.06	0.45	0.31
Ccr6	0.33	-0.65	-0.83
Ccr8	-0.32	0.32	0.84
Cd40lg	-0.32	0.32	0.84

Continuation of Table S1			
Gene Names	ICCD	mCCD	hCCD
Csf1	-0.04	0.42	0.59
Csf2	0.29	0.57	0.62
Csf3	-0.21	0.97	0.43
Cx3cl1	0.10	-0.01	0.05
Cxcl1	-0.32	1.40	1.32
Cxcl10	0.36	2.23	1.65
Cxcl11	-0.32	0.32	0.84
Cxcl12	-0.02	-0.15	-0.19
Cxcl13	-0.40	-0.21	0.45
Cxcl15	-0.46	-0.12	0.84
Cxcl5	0.29	-0.02	0.42
Cxcl9	-0.32	0.32	0.84
Cxcr2	-0.28	0.18	0.86
Cxcr3	-0.27	0.32	0.77
Cxcr5	-0.27	0.50	0.78
Fasl	0.18	0.23	0.50
Ifng	-0.39	0.34	0.84
Il10ra	0.00	0.02	-0.22
Il10rb	0.00	0.08	0.23
Il11	-0.26	0.49	0.80
Il13	-0.45	-0.26	-0.03
Il15	-0.11	0.03	0.00
Il16	0.08	0.16	-0.20
Il17a	-0.32	0.32	0.84
Il17b	0.07	0.34	0.42
Il17f	-0.09	0.29	0.10
Il1a	0.05	0.54	0.87
Il1b	-0.19	2.02	1.59
Il1r1	-0.17	0.14	0.63
Il1rn	0.04	1.60	1.51

Continuation of Table S1			
Gene Names	ICCD	mCCD	hCCD
Il21	-0.32	0.35	0.84
Il27	0.13	0.61	-0.19
Il2rb	0.13	-0.75	-0.36
Il2rg	0.01	0.21	0.66
Il3	-0.32	0.32	0.84
Il33	-0.07	0.06	0.16
Il4	-0.26	0.18	0.20
Il5	0.02	-0.16	0.53
Il5ra	0.32	0.22	0.43
Il6ra	-0.09	-0.03	-0.06
Il6st	-0.02	0.09	0.29
Il7	-0.12	0.25	0.31
Lta	-0.27	-0.15	0.16
Ltb	-0.16	0.93	0.96
Mif	0.03	0.12	0.20
Nampt	0.09	0.00	-0.01
Osm	-0.04	1.14	-0.11
Pf4	0.01	1.00	1.14
Spp1	-0.21	-0.07	0.18
Tnf	-0.05	1.87	2.42
Tnfrsf11b	0.41	0.22	0.56
Tnfsf10	0.03	-0.24	0.01
Tnfsf11	0.22	0.22	0.44
Tnfsf13	-0.02	0.34	0.35
Tnfsf13b	-0.23	0.18	0.34
Tnfsf4	-0.30	0.46	0.73
Vegfa	-0.02	0.28	0.31
End of Table			

Table S2: Average Log2 fold change for all 84 genes 24 hours after BBB opening. Gene expression is shown for the low, medium, and high CCD groups and are relative to the non-sonicated contralateral region. There are 4 mice per CCD group

24 Hours After BBB Opening			
Gene Names	ICCD	mCCD	hCCD
Aimp1	0.71	0.72	0.58
Bmp2	-1.64	-1.88	-1.77
Ccl1	-0.41	-0.07	0.71
Ccl11	0.52	0.44	0.74
Ccl12	0.46	0.56	3.45
Ccl17	0.76	0.53	0.61
Ccl19	0.65	0.60	0.49
Ccl2	0.84	0.61	3.03
Ccl20	-0.29	-0.07	0.71
Ccl22	0.03	0.45	0.95
Ccl24	-0.41	0.00	0.71
Ccl3	-0.04	0.34	2.14
Ccl4	1.92	1.06	3.93
Ccl5	2.12	1.32	2.75
Ccl6	0.40	0.32	0.35
Ccl7	-0.06	0.53	3.10
Ccl8	0.35	0.20	-0.46
Ccl9	0.42	0.38	1.81
Ccr1	-0.08	0.11	0.78
Ccr10	0.31	-0.42	-1.06
Ccr2	0.85	-0.01	0.53
Ccr3	0.58	0.62	0.87
Ccr4	-0.20	-0.03	0.71
Ccr5	0.04	-0.42	-0.06
Ccr6	0.96	0.08	0.15
Ccr8	-0.41	-0.04	0.62
Cd40lg	-0.41	-0.07	0.71

Continuation of Table S2			
Gene Names	ICCD	mCCD	hCCD
Csf1	-0.83	-0.94	-0.41
Csf2	2.31	2.29	2.26
Csf3	-0.04	-0.07	0.67
Cx3cl1	-0.64	-0.77	-0.73
Cxcl1	-0.41	-0.07	0.76
Cxcl10	0.69	-0.82	3.25
Cxcl11	-0.21	-0.07	0.71
Cxcl12	-1.05	-1.15	-1.10
Cxcl13	0.96	1.28	0.69
Cxcl15	1.34	-0.08	0.92
Cxcl5	0.56	0.40	0.03
Cxcl9	-0.41	-0.07	0.71
Cxcr2	0.24	0.21	0.72
Cxcr3	-0.28	-0.38	0.71
Cxcr5	-0.18	0.14	0.53
Fasl	1.49	1.22	1.68
Ifng	-0.41	-0.13	0.60
Il10ra	-1.04	-1.48	-1.44
Il10rb	0.42	0.16	0.13
Il11	0.14	0.15	0.45
Il13	0.41	0.45	1.03
Il15	0.52	0.14	0.40
Il16	-0.09	-0.99	-0.69
Il17a	-0.25	-0.04	0.71
Il17b	0.17	0.98	0.74
Il17f	1.55	1.24	1.20
Il1a	0.70	0.33	0.65
Il1b	0.32	0.34	0.33
Il1r1	-0.35	0.05	0.47
Il1rn	0.77	0.30	1.63

Continuation of Table S2			
Gene Names	ICCD	mCCD	hCCD
Il21	-0.53	-0.07	0.66
Il27	-0.36	0.15	0.30
Il2rb	-0.09	-1.39	-0.08
Il2rg	0.32	-0.01	0.25
Il3	-0.41	-0.07	0.71
Il33	-0.35	-0.52	-0.37
Il4	1.31	1.92	1.20
Il5	0.89	0.73	1.42
Il5ra	1.75	2.04	2.44
Il6ra	-1.28	-1.59	-1.35
Il6st	-0.83	-0.82	-0.74
Il7	0.04	0.43	0.88
Lta	1.30	0.47	0.38
Ltb	-0.29	1.01	0.65
Mif	0.23	0.25	0.26
Nampt	0.40	0.47	0.49
Osm	-0.70	-0.35	0.17
Pf4	1.14	0.99	1.14
Spp1	1.10	0.86	1.06
Tnf	-0.47	-0.43	1.50
Tnfrsf11b	-0.12	-0.29	-0.01
Tnfsf10	-0.48	-0.83	-1.18
Tnfsf11	1.58	0.60	1.66
Tnfsf13	-0.73	-0.93	-0.24
Tnfsf13b	-0.01	0.34	0.02
Tnfsf4	-0.39	-0.07	0.71
Vegfa	0.09	0.11	0.40
End of Table			

Table S3: Average Log2 fold change for all 84 genes 72 hours after BBB opening. Gene expression is shown for the low, medium, and high CCD groups and are relative to the non-sonicated contralateral region. There are 4 mice per CCD group

72 Hours After BBB Opening			
Gene Names	ICCD	mCCD	hCCD
Aimp1	-0.10	-0.06	0.03
Bmp2	0.02	0.45	0.29
Ccl1	-0.75	-0.22	-0.38
Ccl11	-0.06	-0.09	-0.30
Ccl12	0.14	0.27	-0.68
Ccl17	0.14	-0.13	0.15
Ccl19	0.22	0.27	-0.09
Ccl2	0.17	0.77	-0.15
Ccl20	-0.17	-0.46	-0.45
Ccl22	-0.24	-0.03	-0.06
Ccl24	0.08	-0.70	-0.27
Ccl3	-1.51	-0.11	-0.23
Ccl4	0.29	0.01	-0.17
Ccl5	-0.39	0.21	0.08
Ccl6	-1.74	0.00	-0.23
Ccl7	0.25	0.79	-0.15
Ccl8	-0.23	0.46	-1.13
Ccl9	-0.12	-0.03	-0.23
Ccr1	0.02	0.20	-0.16
Ccr10	-1.06	0.26	-0.20
Ccr2	-0.26	0.03	-0.27
Ccr3	-0.10	-0.30	-0.20
Ccr4	0.21	0.17	-0.18
Ccr5	1.52	-0.23	-1.71
Ccr6	0.53	0.01	-0.14
Ccr8	-0.32	0.11	-0.15
Cd40lg	-0.10	0.27	0.26

Continuation of Table S3			
Gene Names	ICCD	mCCD	hCCD
Csf1	-0.29	0.34	-0.12
Csf2	0.09	0.23	-0.03
Csf3	0.13	0.21	-0.43
Cx3cl1	0.36	0.04	0.14
Cxcl1	0.35	-0.19	-0.57
Cxcl10	-0.09	0.00	-0.76
Cxcl11	-0.54	-0.71	-0.01
Cxcl12	0.15	-0.03	-0.08
Cxcl13	-0.93	-0.11	-0.35
Cxcl15	-0.17	0.02	-0.19
Cxcl5	-2.97	-0.97	-1.23
Cxcl9	-0.08	0.15	-0.32
Cxcr2	0.06	0.49	-0.74
Cxcr3	-0.06	0.09	-0.16
Cxcr5	-0.04	0.36	-0.36
Fasl	-0.93	0.14	0.30
Ifng	0.02	0.29	0.04
Il10ra	-0.02	0.19	-0.08
Il10rb	-0.03	-0.02	-0.05
Il11	0.14	-0.06	-0.21
Il13	0.07	0.29	-0.74
Il15	-0.04	0.10	-0.21
Il16	0.19	-0.38	-0.12
Il17a	-0.44	-0.04	-0.14
Il17b	-0.07	-0.23	0.29
Il17f	-0.02	0.03	-0.05
Il1a	0.18	0.13	-0.22
Il1b	0.27	0.17	-0.29
Il1r1	-0.08	-0.08	0.02
Il1rn	-0.13	0.08	-0.26

Continuation of Table S3			
Gene Names	ICCD	mCCD	hCCD
Il21	-0.52	0.19	-0.04
Il27	-0.14	-0.15	-0.56
Il2rb	-0.85	-0.09	0.26
Il2rg	0.26	1.15	-1.07
Il3	0.21	-0.22	-0.18
Il33	-0.30	0.00	0.00
Il4	0.17	-0.09	0.22
Il5	0.09	0.23	-0.11
Il5ra	-0.38	0.12	-0.62
Il6ra	-0.01	0.04	-0.17
Il6st	-0.10	0.03	-0.01
Il7	0.19	-0.21	-0.24
Lta	-0.17	-0.20	-0.06
Ltb	0.05	-0.02	0.00
Mif	0.15	0.00	0.03
Nampt	-0.21	-0.18	-0.14
Osm	0.71	-0.36	-0.54
Pf4	0.07	0.35	-0.33
Spp1	-0.10	0.23	-0.18
Tnf	-0.77	0.48	-0.58
Tnfrsf11b	0.39	-0.03	0.07
Tnfsf10	-0.26	0.29	-0.14
Tnfsf11	0.21	-0.16	-0.18
Tnfsf13	-0.13	0.24	0.02
Tnfsf13b	-0.44	0.21	-0.23
Tnfsf4	-0.64	0.37	-0.27
Vegfa	0.10	0.05	0.02
End of Table			